Title: A novel model for testing the efficiency of removal of calcium hydroxide from complex root canal anatomies

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Running Title: Ca(OH)₂ removal from complex root canal anatomy

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Authorship Declaration

All authors have contributed significantly, and are in agreement with the manuscript.

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Abstract

The purpose of this study was to evaluate the efficacy of several irrigation protocols in the removal of calcium hydroxide [Ca(OH)₂] from simulated internal root resorption cavities in a complex root canal anatomy model. The 20° to 35° curved mesiobuccal roots of 94 maxillary molars were sectioned longitudinally; internal resorption cavities were prepared in the apical third of the canal walls. Calcium hydroxide was placed into the cavities and the root halves reassembled. Four teeth were used as controls, and 90 teeth were randomly divided into 6 experimental groups (n=15), according to the irrigation protocols used: syringe irrigation; H₂O₂ (HP); Navitip FX; Vibringe-syringe; Vibringe-NaviTip FX; ultrasonically activated irrigation (UAI) using an ultrasonic K-file. 2.5% NaOCl and 3% H₂O₂ were used in the HP group while 2.5% NaOCl and 17% EDTA were used in the remaining groups. Stereomicroscope images and radiographs were used to measure the remaining Ca(OH)₂. The model proved to be suitable for simulating complex anatomy. Positive correlation was found between stereomicroscope and radiographic analyses (p<0.05). UAI removed significantly more Ca(OH)₂ than the other experimental groups (p<0.05). The HP group was the least efficient protocol (p<0.05). It would appear that a reliable model has been developed that simulates complex root canal anatomy. Irrigant activation protocols enhanced Ca(OH)₂ removal.

Keywords: Calcium hydroxide; irrigation; NaviTip FX; simulated internal resorption cavity; Vibringe

Introduction

During nonsurgical root canal therapy, the irregular shape and inaccessibility of internal resorption cavities makes such canal irregularities difficult to effectively clean and disinfect (1). Calcium hydroxide [$\text{Ca}(\text{OH})_2$] is recommended in the treatment of internal resorption (1-3), but incomplete removal may interfere with the penetration of root canal sealers into the dentinal tubules (4), reduce the dentine adhesion and sealing ability of root filling materials (5) or may react chemically with the sealer, interfering with its physical properties (6).

Removal of $\text{Ca}(\text{OH})_2$ commonly involves several different irrigants in conjunction with instrumentation (7, 8). However, resorption cavities may be inaccessible to conventional irrigation methods (8, 9) and so different irrigation techniques have been proposed to improve the removal of $\text{Ca}(\text{OH})_2$ from mechanically inaccessible regions of the root canal system (9, 10).

Ultrasonically activated irrigation (UAI) increases the effectiveness of canal disinfection by agitating the irrigation solution inside the canal (11, 12). Although unable to completely remove $\text{Ca}(\text{OH})_2$ from root canals, UAI was better than conventional syringe irrigation (9, 10). The Vibringe system (Vibringe B.V. Corp, Amsterdam, Netherlands) can be used for sonic activation of irrigant solution at low frequency (2-3 kHz) (13) using sonic flow technology combined with acoustic streaming (13, 14). The NaviTip FX (Ultradent, South Jordan, UT) is a #30-gauge (approximately 0.30 mm external diameter and 0.13 mm internal diameter) irrigation needle covered with a brush (15). The NaviTip FX was not totally efficient in producing cleaner root canal walls when used solely as an irrigating needle (15) but did perform well when used with a manual brushing action (16).

Currently, no technique has been able to completely remove $\text{Ca}(\text{OH})_2$ from the root canal system (7-10, 17-24). Few studies have evaluated $\text{Ca}(\text{OH})_2$ removal from root canals with complex anatomy in molar teeth (7, 24), while most studies have used single rooted teeth with single canals (8-10, 17-23). No published study has evaluated $\text{Ca}(\text{OH})_2$ removal from

simulated internal resorption cavities in curved molar roots and the literature is lacking information on the efficacy of NaviTip FX and Vibringe in the removal of $\text{Ca}(\text{OH})_2$ from complex root canal systems. Therefore, the aims of this study were to develop a model simulating complex root canal anatomy, and to evaluate the efficacy of several irrigation protocols in the removal of $\text{Ca}(\text{OH})_2$ from simulated internal root resorption cavities.

Materials and Methods

Specimen Preparation

Following ethics committee approval (GO 16/182), 94 extracted human maxillary molar teeth with mesiobuccal root curvatures of 20° to 35° were selected. Teeth with root caries, cracks or fractures were excluded. The distal and the palatal roots were removed and the teeth were decoronated to obtain a standardized length of 15 mm for each mesial root. Working length (WL) was determined with a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) by subtracting 1 mm from the length when visible at the apical foramen. All root canals were instrumented using rotary nickel-titanium instruments (K3, SybronEndo, West Collins, Orange County, CA, USA) with the sequence of 20.02, 20.04, 25.04, 30.04, 35.04 and 40.04 instruments at WL. Irrigation was with 2 ml of 2.5% sodium hypochlorite (NaOCl) (Werax, İzmir, Turkey) between each instrument. After completion of the preparation, the canals were irrigated with 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) (Werax) for 1 min, followed by 5 ml of 2.5% NaOCl for 1 min. Finally, the root canals were irrigated with 5 ml of distilled water and dried with paper points.

The specimens were then embedded in silicone impression material (Variotime, Heraeus Kulzer GmbH, Hanau, Germany) to create an individual mould for each root. After removal from the mould, grooves were cut with a fine diamond disc (Multicut diamond disc/354, Edenta Ag Dental Products, Hauptstrasse, Switzerland) on the buccal and lingual surfaces without entering the root canal. Then, the blade of a wax carver (Medesy, Maniago, Italy) was wedged into the groove and twisted gently at several spots to achieve splitting of the curved roots. Then, semicircular cavities (1.6 mm diameter) were created in the apical third of the root canal in both root halves using a round bur (801 diamond round bur/016, Edenta Ag Dental Products, Hauptstrasse, Switzerland) (Fig. 1A). The cavities were irrigated with 5 ml of 17% EDTA for 1 min, followed by 5 ml of 2.5% NaOCl for 1 min and finally with 5 ml of distilled water before placing $\text{Ca}(\text{OH})_2$. A $\text{Ca}(\text{OH})_2$ paste (Diapaste, Diadent, Burnaby,

Canada) was injected into the cavities (Fig.1B). Then, the root halves were rejoined and the specimens were positioned in their silicone moulds and a periapical radiograph was taken to confirm the complete filling of the cavities (Fig. 1C). Finally, the remaining coronal canal portion was completely filled with $\text{Ca}(\text{OH})_2$ using a size 40 lentulo spiral bur (Dentsply Maillefer, Ballaigues, Switzerland). The $\text{Ca}(\text{OH})_2$ paste was not applied to the negative control (n=2). It was applied to the positive control (n=2), but no removal procedure was carried out. The orifices of the root canals were sealed with Cavit-W (3M ESPE, Seefeld, Germany) and then the roots were stored at 37 °C at 100% humidity for 2 weeks.

Following the removal of the temporary filling material, the 40/.04 K3 instrument used for root canal preparation was passively introduced to WL by hand to loosen the $\text{Ca}(\text{OH})_2$.

Experimental Procedure

The 90 experimental teeth were randomly divided into six groups (n = 15) according to the $\text{Ca}(\text{OH})_2$ removal protocol used. In all groups the irrigation needle was placed 1 mm short of the WL and the irrigation procedure was applied with up and down motion to produce agitation and prevent binding or wedging of the needle.

Group SI: Irrigation with 5 ml 2.5% NaOCl solution and 5 ml of 17% EDTA for 1 min using a syringe and a 30-G side-vented needle (Maxi-i-probe, Dentsply, Rinn, Elgin, IL).

Group HP: 1 ml 2.5% NaOCl alternated with 1 ml of 3% H_2O_2 to a total of 5 ml of each solution. Each irrigant was applied for 1 min using a syringe and a 30-G side-vented needle.

Group NFX: The same irrigation protocol as in Group 1 but using a syringe and a NaviTip FX needle.

Group VSI: 5 ml 2.5% NaOCl solution and 5 ml of 17% EDTA delivered sequentially using the Vibringe system with a 30-G side-vented needle while sonically activated for 1 minute for each solution.

Group VNFX: The same irrigation protocol was applied as in Group 4 using the Vibringe system with a NaviTip FX needle.

Group UAI: 5 ml 2.5% NaOCl solution and 5 ml of 17% EDTA were delivered into the canal sequentially while activated using an ultrasonic K-file (size 15, 0.02 taper) (Satelec-

Acteon, Merignac, France) coupled to the file-holding adapter of an ultrasonic system handpiece (Satelec P5 Newtron XS, Acteon Group, Merignac, France). The ultrasonic file was placed into the canal 1 mm short of the WL without touching the walls, enabling it to vibrate freely. Each irrigant was passively activated for 1 min at medium power setting. One ultrasonic tip was used for five root canals.

Finally, the root canals were irrigated with 5 ml of distilled water to prevent further irrigant action.

Determination of the amount of remaining $\text{Ca}(\text{OH})_2$

Any remaining $\text{Ca}(\text{OH})_2$ in the artificial resorption cavities was determined using periapical radiographs and a stereomicroscope (Leica MZ16 A, Leica Microsystems, Wetzlar, Germany). Digital radiographs (Digora, Soredex, Helsinki, Finland) from the same angles (vertical and horizontal angles were parallel with the axial and sagittal planes, respectively) were taken of each specimen and coded to prevent identification of the specimens. Each image was analysed using Image J software program (Image J 1.47 V, National Institute of Health, USA). First, the overall area of the resorption cavity visualized in the images was delimited and this measurement corresponded to 100%. Next, the area of the remaining $\text{Ca}(\text{OH})_2$ was measured and the percentage of remaining $\text{Ca}(\text{OH})_2$ was calculated from the ratio between the area of the remaining $\text{Ca}(\text{OH})_2$ and the resorption cavity area. The percentage for each specimen was converted into a score using the following criteria (17):

- 0= The cavity was empty (Fig.2A)
- 1= Less than half of the cavity was filled with $\text{Ca}(\text{OH})_2$ (Fig.2B)
- 2= More than half of the cavity was filled with $\text{Ca}(\text{OH})_2$ (Fig.2C)
- 3= The complete cavity was filled with $\text{Ca}(\text{OH})_2$ (Fig.2D)

Thereafter, the specimens were removed from their silicone moulds and the root halves were separated to evaluate the removal of $\text{Ca}(\text{OH})_2$. Digital images of the internal resorption cavities were captured using a stereomicroscope with x30 magnification. The sequence of all the pictures was randomized and two calibrated endodontists blinded to the groups scored each image using the criteria above.

Statistical Analysis

The kappa test was used to analyse inter-examiner agreement. Differences in $\text{Ca}(\text{OH})_2$

scores among the experimental groups were analysed with Kruskal-Wallis and Mann-Whitney U-tests. The testing was performed at the 95% level of confidence ($p < 0.05$). The correlation between radiographic and stereomicroscopic analysis was statistically analysed using Spearman's correlation test ($p < 0.05$).

Results

Inter-examiner agreement was 81.7%. A positive correlation was found between the results of stereomicroscope and radiographic analyses ($r = 0.865$, $p < 0.001$). The distributions of the scores for the removal of $\text{Ca}(\text{OH})_2$ medicament after stereomicroscope and radiographic analysis are shown in Figure 3. The positive controls showed a score of 3, and the negative controls showed a score of 0 in both analyses.

The stereomicroscope analysis showed that UAI removed significantly more $\text{Ca}(\text{OH})_2$ than the other experimental groups [UAI-SI ($p < 0.001$); UAI-HP ($p < 0.001$); UAI-NFX ($p < 0.001$); UAI-VSI ($p = 0.003$); UAI-VNFX ($p < 0.001$)]. VNFX removed significantly more $\text{Ca}(\text{OH})_2$ than NFX ($p = 0.006$) and SI groups ($p = 0.045$), but was similar to the VSI group ($p > 0.05$). There were no significant differences between the SI, NFX and VSI groups ($p > 0.05$). The HP group was associated significantly less $\text{Ca}(\text{OH})_2$ medicament removal compared with the other experimental groups [HP-SI ($p = 0.008$); HP-NFX ($p = 0.049$); HP-VSI ($p < 0.001$); HP-VNFX ($p < 0.001$); HP-UAI ($p < 0.001$)].

The radiographic analysis revealed that UAI was the most efficient technique in the removal of $\text{Ca}(\text{OH})_2$ [UAI-SI ($p < 0.001$); UAI-HP ($p < 0.001$); UAI-NFX ($p < 0.001$); UAI-VSI ($p = 0.013$); UAI-VNFX ($p = 0.008$)]. VNFX and VSI presented similar results ($p > 0.05$), and both were significantly more efficient than NFX [VNFX-NFX ($p = 0.008$); VSI-NFX ($p = 0.045$)]. The efficacy of SI was similar to NFX, VSI and VNFX ($p > 0.05$). There was no statistical difference between HP and NFX ($p > 0.05$). HP was significantly less efficient in $\text{Ca}(\text{OH})_2$ removal than the remaining experimental groups [HP-SI ($p = 0.010$); HP-VSI ($p = 0.003$); HP-VNFX ($p < 0.001$); HP-UAI ($p < 0.001$)].

Discussion

Techniques previously used to measure $\text{Ca}(\text{OH})_2$ remnants on canal walls include digital photographs (7, 19), stereomicroscopy (9, 10), scanning electron microscopy (20), micro-computed tomography (micro-CT) (24), and spiral CT (25). The model developed for the

present study simulated complex root canal anatomy. Previous studies (7, 24) using similar methodologies considering curvature of roots, tooth selection and apical preparation sizes, did not simulate internal resorption cavities as has been done in the present study. Furthermore, in the studies by Kenee et al (7) and Wiseman et al (24), Ca(OH)_2 was only placed into the root canals at the coronal orifice, while the present study injected Ca(OH)_2 into the resorption cavities deep in the root, and also filled the rest of the canal to ensure the simulated root canal system was completely filled with Ca(OH)_2 .

Comparison of the stereomicroscopic and radiographic analyses allowed testing the validity of radiography to depict the clinical situation, which has not previously been reported in the literature. The significant positive correlation between radiography and direct inspection is clinically relevant and supportive of radiographic confirmation of medicament removal because of the clinical aim of complete Ca(OH)_2 removal from such irregularities. Minuscule amounts of medicament were sometimes not detected in radiographs, and the two dimensional nature of radiographs sometimes created the perception of more material remaining because of their position and orientation. In any case, such small amounts are unlikely to be clinically significant because they should have minimal effect on the seal of the root canal system.

The present study confirmed that UAI was the best technique for the removal of Ca(OH)_2 (7, 17, 19, 20). The higher velocity and volume of irrigant flow with UAI (26) may explain the more effective removal of Ca(OH)_2 in comparison with the other methods. In a previous study, NaviTip FX performed well in cleaning root canal walls of teeth with complex canal morphology presumably because its bristles were able to reach the fins and cul-de-sacs (16). However, NaviTip FX showed similar removal efficacy to the SI group in the present study. When NaviTip FX was used with the Vibringe system, removal efficacy was significantly enhanced although it was not significantly different to the Vibringe system alone.

The effervescence effect of H_2O_2 was expected to enhance the flushing ability of the medicament (27), but the results did not support this supposition. This may be explained by the absence of a chelator in this group. Previous studies have shown that Ca(OH)_2 was not completely removed with 2.25% (28) or 5% NaOCl irrigation alone (4). As chelators appear to be more effective than NaOCl for the removal of Ca(OH)_2 (18), the use of EDTA may have improved the efficacy in the other experimental groups in the present study. This effect may be explained by the formation of complexes between EDTA and calcium ions (18, 21, 25, 29).

The results of the present study indicate that the efficacy of Ca(OH)_2 removal of an irrigation technique may depend not only on mechanical agitation or the flushing ability of the irrigation solutions but also on the chemical activity of the irrigants.

The model developed in the present study produced standard and uniform cavities with well-defined borders. The creation of individual moulds for each specimen provided exact re-approximation of the root halves and formed an enclosed environment representing a more clinically relevant situation. Within the limitations of this in vitro study, irrigant activation techniques, especially UAI, enhanced Ca(OH)_2 removal from complex root canal anatomy. The removal efficacy of Ca(OH)_2 seemed to be dependent on the use of EDTA solution in the irrigation protocol possibly by chelation of the calcium ions but further research is necessary to evaluate the efficacy of different irrigants. Further, based on this work, radiographic analysis may be considered a reliable method for the assessment of Ca(OH)_2 removal.

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Figure Legends

Figure 1: (A) Semicircular cavities in the apical thirds of the root halves; (B) Placement of Ca(OH)_2 into the cavities; (C) Digital radiograph showing the placement of Ca(OH)_2 and reassembling of root halves in the silicone mould.

Figure 2: Representative images of scores (A) Score 0, (B) Score 1, (C) Score 2 and (D) Score 3.

Figure 3: The distribution of scores for the removal of Ca(OH)_2 paste according to the experimental groups. SA: Stereomicroscopic analysis, RA: Radiographic analysis.

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